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Serology of normal primates

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Serological tests for syphilis are of fundamental importance in studying treponemal infections in experimental animals. Non-human primate species have been increasingly employed in treponematology in the past few years. However, Fribourg-Blanc, Niel, and Mollaret (1963, 1966) found an endemic treponeme in cynocephalus monkeys from Guinea. Sepetjian, Guerraz, Salussola, Thivolet, and Monier (1968) noted that 85 per cent. of twenty monkeys from an endemic yaws area in Africa had reactive FTA-200 tests. Furthermore, Kuhn, Brown, and Falcone (1968) observed that 15 per cent. of 250 normal chimpanzees had reactive serum FTA-ABS tests. During the past 6 years, our laboratory has used owl monkeys *(Aotus trivirgatus)*, squirrel monkeys *(Saimiri sciurea)*, and marmoset monkeys *(Tamarinis nigricollis)* in the investigation of ocular and neurosyphilis. The purpose of this report is to review the serological findings in the VDRL, RPR, TPI, FTA Buffered Saline (BS), and FTA-ABS tests in 151 normal primates of these three species.

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Material and methods

Owl monkeys *(Aotus trivirgatus)* and squirrel monkeys *(Saimiri sciurea)* have been the preferred primate species in this laboratory. Both of these monkeys are from South America, and at maturity weigh approximately 0·5 to 1 kg. Both species are relatively hardy, and have survived in good health for over 5 years of laboratory observation. Furthermore, they are inexpensive, and neither has been incriminated as causing an outbreak of disease contagious for man. A number of these monkeys were skin-tested for tuberculosis, and no cases were found.

Upon arrival at the laboratory, each monkey is examined for nasal discharge and diarrhoea. They are placed in separate cages and fed a nonmedicated monkey chow, bananas, and water as desired, and are kept in quarantine for at least 2 weeks before use. Before experimentation, 2–3 ml. blood is drawn aseptically from a femoral vein. The data from these preinoculation serological tests constitute the basis of this report and are summarized in Table I.

All serological tests for syphilis for this laboratory have been performed by Mr. Manuel Sarkar, a technician trained at the National Communicable Disease Center, or by the Venereal Disease Research Laboratory, in Atlanta, or through the gracious cooperation of Dr. Pierre Collart and Dr. A. Fribourg-Blanc, in Paris. The laboratory performing the test is specified in the Table. At the outset, it should be stated that the test results

TABLE I *Serological tests in three species of normal monkeys at three laboratories*

Monkey Species	*Laboratory*	*VDRL*				*FTA-ABS*				*FTA (BS)*			*	*TPI*			
		No	*NR*	*WR*	*R*	*No*	*NR*	±	*R*	*No*	*NR*	±	1+	*No*	*NR*	*AC*	*R*
Owl	Miami	56	56	—	—	56	54	2	—	—	—	—	—	—	—	—	—
	NCDC	24	24	—	—	24	24	—	—	13	2	9	2	5	3	2	—
	F-B	—	—	—	—	4	4	—	—	4	3	·150	—	4	4	—	—
	Total	80	80	—	—	84	82	2	—	17	5	10	2	9	7	2	—
Squirrel	Miami	49	42	4	1	47	43	4	—	—	—	..	—	—	—	—	—
	NCDC	8	5	1	2	8	8	—	—	3	2	—	1	2	1	1	—
	F-B	—	—	—	—	8	8	—	—	8	8	—	—	8	8	—	—
	Total	57	47	5	5	63	59	4	—	11	10	—	1	10	9	1	—
Marmoset	Miami	1	1	—	—	1	1	—	—	—	—	—	—	—	—	—	—
	NCDC	23	23	—	—	23	23	—	—	23	15	3	5*	23	23	—	—
	Total	24	24	—	—	24	24	—	—	23	15	3	5	23	23	—	—

NCDC = National Communicable Disease Center, Venereal Disease Research Laboratory, Atlanta, Georgia
F-B = Dr. A. Fribourg-Blanc, Paris, France
*1+ = 1+ or more on FTA (BS) test in marmoset monkeys

from the four laboratories were consistently in good agreement. The only exception was a more frequent report of 'anticomplementary' TPI tests on squirrel monkey sera in Atlanta than in Paris.

Findings (Table II)

OWL MONKEY

VDRL tests were performed on eighty normal (preinoculated) owl monkeys and were nonreactive in each instance. Clark, Yobs, and Artley (1968) found all of 37 owl monkeys nonreactive to the VDRL test. Therefore, on the basis of a combined experience with 117 normal owl monkeys, 100 per cent. of this species is nonreactive to the VDRL test.

FTA–ABS tests were performed on 84 normal owl monkey sera. 82 of these were nonreactive. Two of the sera were reported as having equivocal (+–NR) reactivity. The Atlanta series—Clark, Yobs, and Artley (1968)—reported all of 29 normal owl monkey sera as nonreactive to the FTA–ABS test. More than 98 per cent. of owl monkey sera in both series were nonreactive to the FTA–ABS test.

The FTA (BS) test is the fluorescent treponemal antibody test done in buffered saline and without absorption. This test is now under evaluation by the Venereal Disease Research Laboratory. Of thirteen normal owl monkey sera tested by the FTA (BS) test in Atlanta, the results were nonreactive in two, ± nonreactive in nine, and 1+ reactive in two. Of four other sera tested in Paris, three were nonreactive, and one showed a low titre (1:150) by this method. Four normal human sera studied in Atlanta were reported as 1+ to the FTA (BS) test, but nonreactive to the FTA–ABS test. The significance of equivocal or low titred responses to the FTA (BS) test has not been determined. The test is less specific than the FTA–ABS test. The data are presented as recorded.

TABLE II *Serological tests in normal monkeys*

Test	*Result (per cent.)*	*Monkey*		
		Owl	*Squirrel*	*Marmoset*
VDRL	Nonreactive	100	86	100
	Weakly reactive	0	7	0
	Reactive	0	7	0
FTA–ABS	Nonreactive	98	93	100
	Equivocal	2	7	0
	Reactive	0	0	0
TPI	Nonreactive	100		100
	Reactive	0		0

The RPR (rapid plasma reagin) test was performed by Mr. Sarkar on eight normal owl monkey sera, and was nonreactive in each instance.

Because of the time and expense involved in performing the TPI test, only eight normal owl monkey sera were evaluated. All of these were nonreactive. All 29 sera in the Atlanta series were nonreactive to the TPI test. Therefore, 100 per cent. of 37 normal owl monkey sera gave nonreactive results to the TPI test.

SQUIRREL MONKEY

VDRL tests were performed on sera from 57 normal squirrel monkeys. The test was nonreactive in 47 sera, weakly reactive in five, and reactive in five. Therefore, 17 per cent. of normal squirrel monkeys showed at least some degree of reagin reactivity in the serum to the VDRL test.. This stood in definite contrast to the results found in *Aotus*, where the VDRL test was nonreactive in 100 per cent. of 117 normal owl monkey sera.

FTA–ABS tests were performed on sera from 63 normal squirrel monkeys. The test was nonreactive in 59, and equivocal (+–NR) in four sera. Therefore, the FTA–ABS test gave an equivocal result in 6 per cent., a nonreactive result in 94 per cent., and in no instance was even a 1+ positive FTA–ABS test found.

The FTA (BS) test was done on eleven normal squirrel monkey sera. Ten of these were nonreactive, and one gave a 1+ reactive test in buffered saline.

Rapid plasma reagin (RPR) tests were performed in this laboratory on 22 normal squirrel monkey sera, and were nonreactive in each instance.

TPI tests were performed on ten normal squirrel monkey sera. One of these was reported as anticomplementary, and nine were nonreactive. Anticomplementary TPI reports were not uncommon in the squirrel monkey, particularly in inoculated animals, when reported from the Atlanta laboratory. This point merits further investigation.

MARMOSET MONKEY

A small series of marmoset monkeys was studied. In 24 normal sera from *Tamarinis nigricollis*, VDRL, TPI, and FTA–ABS tests were nonreactive in each instance. The less specific FTA (BS) test was completely nonreactive in only fifteen, however, and at least equivocal reactivity was found with this test in eight normal marmoset sera. The significance of this remains to be determined.

Although serologically desirable with regard to the VDRL, TPI, and FTA–ABS tests, marmosets proved to be a less desirable species for neuro-ophthalmological research than *Aotus* or *Saimiri*. Marmosets have very small eyes, and often went into shock after only 2 to 3 ml. of blood was drawn. Furthermore,

these animals have an ill-tempered disposition, and were frequently found to be infested with internal parasites. One marmoset was inoculated intradermally with *T. pallidum* from a human chancre, and showed seroconversion to VDRL, TPI, and FTA–ABS tests at 3 months, but died shortly thereafter. For all these reasons, further use of this species was discontinued.

Discussion

Inability to maintain virulent *Treponema pallidum* in artificial media has necessitated the use of animal inoculations in studying experimental treponemal infections. Rabbits are most commonly employed in this research, but approximately 25 per cent. of normal rabbits show at least a weakly reactive serum reagin (VDRL) test (Pannu, Rosenberg, Israel, and Smith, 1967). Furthermore, rabbits are susceptible to an endemic treponemal infection with *T. cuniculi* which cannot be morphologically or serologically differentiated from *T. pallidum* (Smith and Pesetsky, 1967). It was recently reported that the VDRL test showed at least some degree of reactivity in 18·8 per cent. of albino rabbits, but in 38·6 per cent. of pigmented rabbits (Levine, Smith, Martinez, Rios Montenegro, and Nicol, 1969). An interesting report bearing on the latter point was the study by Frazier and Mu (1930). An emulsion of virulent Nichols strain *T. pallidum* was inoculated at the same time and in equal dosage into the testes of eight albino and nine brown rabbits. These authors noted more pronounced orchitis in the albino rabbits, and 85·7 per cent. of the albino rabbits developed metastatic eye lesions. The brown rabbits showed a primary response which was less pronounced, both in magnitude and duration of orchitis, and only 12·5 per cent. manifested ocular lesions. Frazier and Mu concluded: 'The evidence indicates that, under the conditions of the experiment, the albino rabbits offered less resistance to infection with *Treponema pallidum* than did the brown breed of rabbit'. They further emphasized: 'These divergent reactions must be attributed to some inherent constitutional factor of the host and not to strain specificity of the infecting organism'. A strong plea was recently made for the rabbit infectivity test in syphilis (Turner, Hardy, and Newman, 1969). These authors pointed out the possibility that treponemes might remain in the hosts for many years, but noted that many of the so-called 'L' forms are essentially intracellular parasites. Although the organisms are commonly located extracellularly, several observers have reported the presence of intracellular *T. pallidum* (Bandi, 1906; Sauvage and Levaditi, 1906; Nyka, 1934; Nicol, Rios Montenegro, and Smith, 1969). It is important to note that this question has now been resolved beyond any reasonable doubt by the detailed electron microscopical studies of intracellular *T. pallidum* recently reported by Ovcinnikov and Delektorskij (1969).

Studies of experimental syphilis have been performed in animal species other than rabbits rather infrequently. Rodents, such as the hamster, are susceptible to treponemal infection, but do not show late lesions in which the clinical investigator is interested when following the natural course of the disease in man. Sheep and dogs were successfully inoculated with *T. pallidum* (Bertarelli, 1906) and Levaditi and Yamanouchi (1908) reported lesions after inoculating five calves with massive numbers of *T. pallidum* organisms.

The first successful positive transfer of syphilis to the experimental primate was reported by Metchnikoff and Roux (1906). The *Macaques zati* was the first animal ever infected with *T. pallidum* to be reported in the literature. After many successful inoculations, these authors discontinued using this primate because of the high incidence of tuberculosis in the species (Metchnikoff and Roux, 1906).

Turner and Hollander (1957) reported the successful transfer of Nichols strain *T. pallidum* to the African green monkey and the Rhesus monkey. The initial expense of the animals was the reason cited for not continuing with nonhuman primates as a research animal.

Fribourg-Blanc, Niel, and Mollaret (1963) have reported a treponeme endemic in the cynocephalus monkey from Guinea. They found 65 per cent. of eleven such monkeys to have reactive TPI tests. They also found that cynocephalus monkeys from Kenya showed no reactive TPI tests. In 1966 these authors reported that all of 1,168 cynocephalus monkeys from Cambodia were nonreactive to the TPI test.

Sepetjian and others (1968) stated that 85 per cent. of twenty monkeys from an endemic yaws area in Africa had reactive FTA-200 tests, but they also found that all of 91 monkeys' sera from an endemic syphilis area were nonreactive to the FTA–200 test. Kuhn, Brown, and Falcone (1968) reported that 15 per cent. of 250 normal chimpanzees had reactive FTA–ABS tests. Elsas, Smith, Israel, and Gager (1968) noted that not a single reactive FTA–ABS test had been encountered in a normal owl monkey in their investigations.

For over 6 years, this laboratory has studied experimental syphilis in three primate species. It is concluded that, because of its virtually 100 per cent. nonreactive serological picture, as well as its size, cost, powers of survival in captivity, infectivity, and large eye-balls, the owl monkey is a worth-while ex-

perimental animal for the investigation of ocular and neurosyphilis.

Summary

A serological investigation of the VDRL, TPI, and FTA-ABS tests in normal owl monkeys, squirrel monkeys, and marmoset monkeys has been performed. Reasons for preference of the *Aotus trivirgatus* as an experimental primate suitable for the study of ocular and neurosyphilis include the fact that 100 per cent. of a series of normal owl monkeys were sero-negative to both VDRL and TPI tests.

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La sérologie des primates normaux

SOMMAIRE

On a pratiqué une enquête sérologique avec les épreuves VDRL, TPI et FTA-ABS chez des singes-hiboux, des singes-écureuils et des ouistitis. Les raisons de préférer l'*Aotus trivirgatus* (singe-hibou) comme primate d'expérience convenant à l'étude de la syphilis oculaire et nerveuse, comprend le fait que 100% d'une série de singes-hiboux fut séronégative et au VDRL et au TPI.

Addendum

Since this manuscript was submitted, Dr. Jerome Goldman of Washington, D.C., has mentioned in a preliminary communication that electron microscopic studies in his institution appeared to confirm the intracellular presence of *Treponema pallidum*. Furthermore, electron microscopic confirmation of the intracellular presence of *Treponema pallidum* was definitely reported from Paris in the June 1, 1970, issue of *La Revue de Médecine*.